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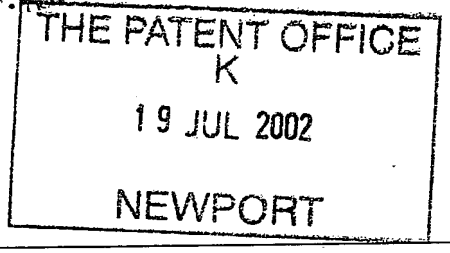

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1. Your reference — ARB/P/201/GBA

2. Patent application number *(The Patent Office will fill in this part)* — 0216780.7

19JUL02 E734527-1 010100
P01/7700 0.00-0216780.7

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Bradford Particle Design Ltd
Unit 69 Listerhills Science Park
Campus Road
Bradford
BD7 1HR
GB

19 JUL 2002

Patents ADP number *(if you know it)* 8377814001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

Methods of particle formation

5. Name of your agent *(if you have one)*

Andrea R. Brewster et al.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Greaves Brewster
24A Woodborough Road
Winscombe
North Somerset
BS25 1AD

Patents ADP number *(if you know it)* 7869829002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

Yes

Methods of particle formation

Field of the invention

The present invention relates to methods for forming particles of target substances and to particulate products of the methods.

Background to the invention

Polyalkylene glycols, in particular polyethylene glycol (PEG, also known as polyethylene oxide (PEO) or polyoxyethylene (POE) or polyether glycol) are widely used as excipients in pharmaceutical formulations since they can help to enhance the water solubility, and hence bioavailability on administration, of pharmaceutically active materials. PEG in particular has low toxicity and immunogenicity and is particularly well known as an excipient for use with proteinaceous actives, for which it can help to improve shelf stability and also increase the half-life *in vivo*.

Polyalkylene glycols such as PEGs can be coformulated with active substances for instance as physical mixtures or as intimate solid dispersions. It is also possible, however, to conjugate a polyalkylene glycol (hereafter PAG) covalently to an active substance. This is achieved by "activating" the PAG, typically by replacing a terminal hydroxy group with a reactive functional group suitable for conjugation to the relevant active substance. The preparation of such "activated" PAGs, and their conjugation to active substances, are disclosed for example in EP-1 176 160, US-6,214,966, US-6,258,351, WO-99/45964, WO-01/00246, WO-01/24831, WO-01/26692, WO-01/45796, WO-01/46291, WO-01/47562, WO-01/62299 and WO-01/62827, with particular emphasis on PEGs.

Using such technology, significant difficulties can be experienced in isolating the activated PAG product. Typically it is precipitated, following its synthesis, from an organic solvent such as dichloromethane, following which solvent evaporation and drying steps are required in order to achieve a particulate solid product. This can be a lengthy process; the precipitate may take a long time to settle following centrifugation and often long drying periods (for instance 2 to 4 days' air

drying) are needed. The result may be a coarse powder or flakes instead of the fast dissolving, fine particulate which would be more desirable for future processing. The product may also contain undesirably high levels of residual solvent, and the process itself requires high levels of organic solvents which must subsequently be disposed of. Yields, furthermore, tend to be low.

It would be preferable if activated PAGs could be more easily converted into a dry particulate product, or still more preferably be prepared directly in particulate form.

Active substances such as proteins, peptides and small molecule pharmaceuticals may be conjugated with such activated PAGs to enhance their aqueous solubility, extend their circulating half life and improve their bioavailability and/or stability especially at high concentrations. This is of particular use in preparing injectable drug formulations. However, the resultant conjugate tends to be synthesised in aqueous solution, and if stored in this form lacks long-term stability, typically needing to be refrigerated and having a shelf life of only about 30 days. When prepared in particulate form (for instance by solvent extraction and drying), again poor handling properties and high residual solvent levels can result, yields are often low and processing lengthy. Again it would be preferable if the conjugates could be prepared in, or converted into, a particulate form with improved physicochemical properties, which could be stored dry and re-hydrated, without undue loss of activity, prior to use.

A number of techniques are known for forming particles of a substance of interest (a "target substance"). Amongst them are processes which make use of supercritical or near-critical fluids as anti-solvents. This technique is generally known as GAS (Gas Anti-Solvent) and involves contacting a solution or suspension of the target substance, in a suitable fluid vehicle, with a supercritical or near-critical fluid (SCF/NCF) which is a nonsolvent for the substance but is miscible with the vehicle. The SCF/NCF, referred to in this context as an "anti-solvent", extracts the vehicle and thus causes precipitation of the target substance.

A supercritical fluid is a fluid which is simultaneously above both its critical temperature T_c and its critical pressure P_c. "Near-critical fluid" is here used to refer to a fluid which is either (a) above its T_c but slightly below its P_c, (b) above its P_c but slightly below its T_c or (c) slightly below both its T_c and its P_c.

The basic GAS technique is described in Gallagher et al, "Supercritical Fluid Science and Technology", *ACS Symp. Ser.*, 406, p334 (1989) and is exemplified for instance in WO-90/03782. Other versions of the technique are known, typically involving different ways of contacting the reagent fluids. These include "ASES" (Aerosol Solvent Extraction System – see US-5,043,280), PCA (Precipitation using Compressed fluid Anti-solvent – see eg, Dixon et al, *AIChE Journal*, 1993, 26, 127), SAS (Solvent Anti-Solvent – see eg, Yeo et al, *Biotechnology and Bioengineering*, 1993, 41, 341) and SEDS™ (Solution Enhanced Dispersion by Supercritical fluid – see WO-95/01221, WO-96/00610, WO-98/36825, WO-99/44733, WO-99/59710, WO-01/03821 and WO-01/15664, our co-pending PCT patent application no. PCT/GB PCT/GB01/04873 and our co-pending UK patent application no. 0117696.5).

Such techniques have enabled the formation of particulate products with desirable physicochemical characteristics (in particular particle size and size uniformity), often with a high degree of control over aspects such as the purity, morphology and yield of the product. This has seen wide application in the production of pharmaceutically active substances and their excipients.

There are considerable advantages to using SCF/NCF anti-solvents, in particular the wide variations in their solvent power which can be achieved by relatively small adjustments in their temperature and pressure. The most commonly used SCF/NCF is CO_2, which is non-toxic, non-flammable, generally inert and also relatively inexpensive. It also has a relatively low T_c (31 °C/304 K) and P_c (74 bar).

Nevertheless, not all substances are suitable for processing using supercritical or near-critical fluids. Some are unable to withstand the processing temperatures needed in order to maintain the anti-solvent fluid in its supercritical or near-critical state. This may be the case, for instance, with certain biologically active materials such as proteins, peptides, nucleic acids and their derivatives. It is also the case for certain polymers which "melt", or form glasses, at the required operating temperatures. This latter problem can be exacerbated if the anti-solvent itself (as in the case of CO_2 for instance) acts to lower the glass transition temperature of the polymer.

PAGs such as PEG and polypropylene glycol are examples of such polymers. Dependent on their molecular weight, PEGs for instance tend to melt at temperatures between about 30 and 45 °C,

sometimes even below room temperature, and are therefore unsuitable for processing with supercritical or even near-critical CO_2.

Statements of the invention

It has surprisingly been found, despite the above, that PAGs (in particular PEGs) and their derivatives, and formulations containing them, may be processed by modifying the basic GAS technique, in order to obtain the above described advantages of the GAS process despite the apparent incompatibility of these polymers with supercritical fluid processing.

According to a first aspect of the present invention there is provided a method for forming particles of a target substance which comprises a polyalkylene glycol (PAG) or a derivative or conjugate thereof, which method comprises carrying out a GAS process, preferably a SEDS™ process, on a solution or suspension of the target substance in a fluid vehicle (the "target solution/suspension"), but using as the anti-solvent fluid a compressed fluid which at the point of its contact with the target solution/suspension is at a temperature of 25 °C (298 K) or below.

The target substance may in particular comprise a polyethylene glycol (PEG), a polypropylene glycol (PPG), a copolymer of the two, a derivative or conjugate of any of these or a mixture of any of them. More particularly it comprises a PEG or a derivative or conjugate thereof.

Carrying out a GAS process on the target solution/suspension involves contacting it with a compressed fluid anti-solvent under conditions which allow the anti-solvent to extract the vehicle from the target solution/suspension and to cause particles of the target substance to precipitate from it. The conditions should be such that the fluid mixture thus formed between the anti-solvent and the extracted vehicle is still in a compressed state. The anti-solvent fluid should be a nonsolvent for the target substance and be miscible with the fluid vehicle.

Carrying out a SEDS™ process involves carrying out the above described GAS process, but using the anti-solvent fluid simultaneously both to extract the vehicle from, and to disperse, the target solution/suspension. In other words, the fluids are contacted with one another in such a manner that the mechanical (kinetic) energy of the anti-solvent can act to disperse the target solution/suspension at the same time as it extracts the vehicle. "Disperse" in this context refers generally to the transfer of kinetic energy from one fluid to another, usually implying the

formation of droplets, or of other analogous fluid elements, of the fluid to which the kinetic energy is transferred.

Suitable SEDS™ processes are described in WO-95/01221, WO-96/00610, WO-98/36825, WO-99/44733 and WO-99/59710, WO-01/03821 and WO-01/15664, in our co-pending PCT patent application no. PCT/GB PCT/GB01/04873 and in our co-pending UK patent application no. 0117696.5; these may be used in the method of the present invention provided the anti-solvent is a compressed fluid at a temperature of 25 °C or below. In particular, the target solution/suspension and the anti-solvent are preferably contacted with one another in the manner described in WO-95/01221 and/or WO-96/00610, being co-introduced into a particle formation vessel using a fluid inlet means which allows the mechanical energy (typically the shearing action) of the anti-solvent flow to facilitate intimate mixing and dispersion of the fluids at the point where they meet. The target solution/suspension and the anti-solvent preferably meet and enter the particle formation vessel at substantially the same point, for instance via separate passages of a multi-passage coaxial nozzle.

References in this specification to a fluid entering a vessel are to the fluid exiting an inlet means (for example, a nozzle) used to introduce the fluid into the vessel. For these purposes, therefore, the inlet means is to be considered as *upstream of* the vessel in the direction of fluid flow, although parts of it (in particular its outlet) may be located physically within the vessel.

Other suitable SEDS™ processes are disclosed in WO-99/52507, WO-99/52550, WO-00/30612, WO-00/30613 and WO-00/67892.

In the context of the present invention, "compressed" means, at any given temperature, above the vapour pressure of the fluid concerned, preferably above atmospheric pressure, more preferably from 70 to 200 bar or from 80 to 150 bar.

More preferably "compressed" means above the critical pressure P_c for the fluid or fluid mixture concerned. In practice, the pressure is likely to be in the range $(1.01 - 9.0)P_c$, preferably $(1.01 - 7.0)P_c$.

"PAG or PAG derivative" for the purposes of this invention includes any polymeric molecule containing in its polymeric backbone the alkylene glycol repeat unit $(O(CH_2)_n)$, where n is an

integer from 2 to 8, preferably 2 or 3 (ethylene glycol or propylene glycol), more preferably 2. Such a molecule preferably contains $(O(CH_2)_n)_m$ where m is an integer from 40 to 3,000, typically from 100 to 2,000, more typically from 100 to 1,000 or from 200 to 1,000. The term "PAG or PAG derivative" includes any polymeric molecule in which at least 50 %, preferably at least 70 %, more preferably at least 80 % or at least 90 %, of the repeat units (regardless of the positions of those repeat units, and therefore of the structure (eg, linear or branched) of the polymer) are alkylene glycol units, preferably units of the formula $(O(CH_2)_n)$ where n is as defined above. The term therefore encompasses copolymers and terpolymers (in each case either random or block) containing (i) two or more different alkylene glycol repeat units and/or (ii) other repeat units in addition to the alkylene glycol units.

PAG as used herein is meant to encompass (i) linear PAGs (both monofunctional and difunctional or "dumbell"-like), (ii) branched PAGs having more than one PAG chain extending from a central core structure, for instance those having the general formula $T-(PAG)_p$ where T is a multivalent "core" group such as pentaerythritol, lysine or glycerol, PAG is a polymeric chain containing the alkylene glycol repeat unit and p represents the number of arms and is typically from 2 to 120, (iii) pendant PAGs having reactive functional groups (for instance carboxyl groups) along the polymer backbone as well as or instead of at their terminae, (iv) forked PAGs represented by the formula $PAG-(V)_r$ where V is a group suitable for attachment to another moiety and r is an integer of 2 or greater, (iv) dendritic PAGs having highly branched structures, and the like. The term "PAG derivative" may be construed accordingly. Branched PAGs and their derivatives may for instance be prepared by addition of an alkylene oxide to various polyols, such as glycerol, pentaerythritol and sorbitol, the polyol representing the multivalent "core".

Where a branched PAG or PAG derivative includes propylene glycol or higher alkylene glycol repeat units, as in polypropylene glycol, its conformation may be isotactic, syndiotactic or atactic. Isotactic polymers tend to have relatively high levels of crystallinity and melting points, syndiotactic polymers intermediate melting points and partial crystallinity and atactic polymers relatively low melting points and amorphous natures. The present invention may thus be of particular use in processing syndiotactic and especially atactic polymers.

A PAG or PAG derivative may be cross-linked, for instance as described in US-6,258,351 or WO-01/00246.

"PAG derivative" is meant to encompass polymers having any of a number of suitable functional groups attached to the polymeric backbone or more preferably at its terminae, depending upon its structure and intended use. A PAG derivative may therefore be monofunctional (ie, having one reactive functional group (or precursor thereof) suitable, eg, for attachment to a drug moiety, and one inert or relatively unreactive terminus such as methyl), bifunctional (having two reactive functional groups or precursors thereof for reaction with two other moieties, wherein the functional groups may be the same or different), multifunctional, etc. Additionally, a PAG derivative may contain one or more spacer or linker groups (these terms may by used interchangeably) separating the main polymer portion of the molecule from a reactive terminus. PAGs and PAG derivatives may also contain one or more hydrolysable functionalities within the polymer chain for in-vivo hydrolysis of portions of the polymer chain.

According to the first aspect of the invention, the target substance is typically either

(a) a polyalkylene glycol (PAG) of the general formula (I):

$$R^1\text{-}(O(CH_2)_n)_m\text{-}O\text{-}R^2 \qquad (I)$$

where n and m are integers as defined above, and R^1 and R^2 are each independently either hydrogen or an unreactive end group such as alkyl, in particular methyl or ethyl; or

(b) a PAG derivative, as defined above, which contains alkylene glycol repeat units and more preferably contains the group $(O(CH_2)_n)_m$ where n and m are as defined above, together with one or more functional groups on the main polymer chain(s) and/or at its terminae; or

(c) a PAG conjugate, which comprises a PAG derivative as defined above, covalently bound to one or more active substances.

The PAG, PAG derivative or PAG conjugate is preferably hydrophilic.

The PAG derivative may be an "activated PAG" containing one or more reactive functional groups X on the main polymer chain(s) and/or at its terminae, where X is an "activating" group selected to facilitate subsequent conjugation of the polymer to an active substance such as a protein or peptide. Examples of such activated PAGs are well known and are disclosed, for

instance, in EP-1 176 160, US-6,214,966, US-6,258,351, WO-99/45964, WO-01/00246, WO-01/24831, WO-01/26692, WO-01/45796, WO-01/46291, WO-01/47562, WO-01/62299 and WO-01/62827.

In particular an activated PAG may have the general formula (II):

$$R^1\text{-}(O(CH_2)_n)_m\text{-}X \qquad \text{(II)},$$

where R^1, X, n and m are as defined above.

X comprises a functional group which is able to react with a group on another molecule such as a drug, targeting moiety or the like. Suitable functional groups include (a) sulphones, in particular vinyl sulphones such as SO_2-CH=CH_2 and active alkyl (in particular ethyl) sulphones such as SO_2-CH_2-CH_2-L_e where L_e is a leaving group such as halide, in particular chloride; (b) active esters, such as carboxylic acid esters, carbonate esters, sulphonate esters, isocyanates, isothiocyanates, acrylates, methacrylates, benzotriazolyl esters or succinimidyl (preferably carboxylate or carbonate) esters, examples being N-hydroxysuccinimidyl esters or N-sulphosuccinimidyl esters; (c) acetals, diones, aldehydes and aldehyde hydrates; (d) epoxides; (e) amines, in particular NH_2; (f) alcohols and thiols; (g) maleimides, for instance N-maleimides as described in WO-01/62827; (h) hydrazides; (i) dithiopyridines and vinyl pyridines; (j) iodoacetamides; (k) carbamate (in particular carbamide) precursors of the general formula –O-C(O)-X′, where X′ is a reactive group such as halide, hydroxyl, 1-benzotriazolyloxy, p-nitrophenyloxy, 1-imidazolyl, N-sulphosuccinimidyloxy or especially N-succinimidyloxy, preferred versions being of the form –Ar-O-C(O)-X where Ar is an aromatic group; (l) carboxylic acids; (m) mesylates, tosylates or tresylates; (n) alkenyls; (o) acrylamides; (p) glyoxals; (q) halides; and (r) alkoxy groups or hydroxyl.

Other suitable "activated PEGs" of this type are described in the 2001 catalogue of Shearwater Corporation (Huntsville, Alabama, US) entitled "Polyethylene Glycol and Derivatives for Biomedical Applications". Preferred groups X are active esters such as N-hydroxysuccinimidyl (NHS) esters, aldehydes and aldehyde precursors such as acetals, maleimide, benzotriazole carbonate and carboxyl.

The activated PAG may include a linking group L_i by which the reactive group X is bound to the rest of the polymer. Generally L_i may be either hydrolytically stable or unstable, depending on the intended use of the derivative. Suitable hydrolytically unstable (ie, hydrolysable) linking groups can include those containing carboxylate esters, phosphate esters, orthoesters, anhydrides, acetals, ketals, imines, ester-linked amino acids, peptides or oligonucleotides, for instance as described in US-6,214,966 or US-6,258,351. Hydrolytically stable linking groups can include groups containing optionally substituted alkylene or alkenylene (preferably C_1-C_6) groups (that may be branched or straight-chain), ethers, thioethers, amines, amides, imides, carbamides and certain esters. Photolytically degradable linking groups include cinnamate dimers and cinnamylidine dimers. The linking group may carry substituents (for instance, sterically hindering groups) to influence its stability.

The linking group may itself contain active moieties, for example amino groups, through which the L_i-polymer attachment may be formed.

The group X may be multi-functional, ie, reactive at two or more sites. The linking group L_i, if present, may be or comprise a multivalent group (for instance CH, the carbon atom of which may form attachments to 1, 2 or 3 other atoms depending on its degree of saturation) and be linked to more than one reactive group X and/or to more than one group R^1-$(O(CH_2)_n)_m$. A further link (for example a C_1-C_6 alkylene or alkenylene group) may be present between each group X and the multivalent group L_i.

"Activated" PAG derivatives containing a terminal group X may for example be synthesised as described in EP-1 176 160, US-6,214,966, US-6,258,351, WO-99/45964, WO-01/00246, WO-01/24831, WO-01/26692, WO-01/45796, WO-01/46291, WO-01/47562, WO-01/62299 and/or WO-01/62827, by appropriate reactions at the terminal –OH group of a PAG base polymer.

In accordance with the present invention, a PAG conjugate is an "activated PAG" of the type described above, which has been conjugated by means of a covalent bond to an active substance. It is typically a PAG-active substance conjugate of the general formula (III):

$$R^1\text{-}(O(CH_2)_n)_m\text{-}Y\text{-}Z \qquad \text{(III)},$$

where Z is an active substance for instance as referred to in EP-1 176 160, US-6,214,966, US-6,258,351, WO-99/45964, WO-01/00246, WO-01/24831, WO-01/26692, WO-01/45796, WO-01/46291, WO-01/47562, WO-01/62299 and/or WO-01/62827, and Y represents a covalent link formed between an activating group X (optionally with a linking group L_i) as defined above and a reactive group in the active substance. The active substance may in particular be a pharmaceutically or nutriceutically active substance. More particularly it is a macromolecular substance such as a protein or peptide (including enzymes, hormones, antibodies and antigens) or nucleic acid. Other potential active substances include nucleotides, nucleosides, vitamins, amino acids, lipids including phospholipids and aminolipids, carbohydrates such as polysaccharides, cells, viruses, as well as small molecule (ie, non-macromolecule based) actives such as pharmaceuticals and imaging agents.

The conjugation of such actives with an activated PAG may be used to alter their aqueous solubilities and/or to achieve other effects such as improved stability, reduced toxicity or controlled release on administration. Such PAG conjugates may be suitable for delivery in the form of a solution or suspension, preferably an aqueous solution, such as by injection, orally or by any other suitable route.

The "active substance" may comprise a substrate on which the PAG derivative is to be immobilised. It may be in any appropriate physical form, for instance microparticles, liposomes or micelles.

Where the group X is multi-functional, or the activated PAG derivative carries more than one group X, the PAG conjugate may contain a corresponding number of active substance moieties.

Preferably the active substance Z in the conjugate is a protein containing for example:

a) a thiol group –SH (which may itself be formed by reduction of a disulphide bond S-S), such as in a cystine moiety – this may be conjugated to for instance a sulphone group;

b) an amino group $-NH_2$, such as in a lysine moiety – this may be conjugated to for instance a sulphone, carboxylic acid, active ester (eg, carboxylate, carbonate, sulphonate or isocyanate), aldehyde or aldehyde hydrate, epoxide, mesylate, tosylate, tresylate or carbamide precursor group;

c) an imino group –NH, such as in a histidine moiety – this may be conjugated to for instance a sulphone group; or

d) an acid group $-CO_2H$ – this may be conjugated to for instance a hydroxyl or amine group.

Thus, the active substance may be conjugated to the PAG by means of, without limitation, a (preferably hydrolytically stable) group such as an amide, urethane, amine, imine, carbamate, thioester, ester, carbonate ester, thioether or urea.

The active substance may be conjugated to more than one PAG group R^1-$(O(CH_2)_n)_m$ via corresponding Y groups.

A PAG derivative or conjugate may have the general formula (IV):

$$D\text{-}(O(CH_2)_n)_m\text{-}D' \qquad \text{(IV)},$$

where D and D′ are each independently either X or Y-Z (optionally with linking group(s) L_i) as defined above.

A non-linear PAG derivative or conjugate may naturally incorporate more than one group X or Y-Z.

In particular where the PAG or PAG derivative is a PEG or PEG derivative, or a PPG or PPG derivative, its preferred molecular weight is from 2 to 60 kDaltons, more preferably from 2.5 to 40 kDaltons, still more preferably from 5 to 30 or from 10 to 30 kDaltons, suitably at least 5 kDaltons. Typically, the lower the molecular weight, the lower the preferred operating temperature in the method of the invention, since PAG solubility (for instance in the anti-solvent fluid) tends to increase with decreasing molecular weight. Thus, for instance, for a PEG having a molecular weight of at least 5, preferably at least 10, kDaltons, an operating temperature of 25 °C or lower may be used; for a PEG with a molecular weight below 5 kDaltons, it may be preferable to work at lower temperatures such as between 0 and 10 °C, more preferably between 0 and 5 °C.

The method of the invention is particularly suitable when the PAG, PAG derivative or PAG conjugate melts or otherwise degrades (by which is meant undergoes any undesired change in physicochemical form and/or properties) at temperatures above 30 or preferably above 25 °C. (The term "melts" includes glass formation.) The PAG or derivative or conjugate may for example have a low glass transition temperature T_g under the operating conditions used (taking into account if necessary the other reagents present, since CO_2 for instance can lower the T_g of, and/or "swell", polymers such as PEG). A low polymer T_g might typically be 30 °C or below, 25 °C or below or 20 °C or below. Polymers suitable for processing using the present invention might have melting points of 70 °C or lower, possibly 65 °C or 60 °C or 55 °C or lower.

In the method of the invention, the target substance preferably comprises no less than 10 % w/w of a PAG, PAG derivative and/or PAG conjugate, preferably at least 20 % w/w, more preferably at least 30 % w/w or 40 % w/w or 50 % w/w or 60 % w/w or 70 % w/w.

Generally speaking, in the method of the invention the temperature at the point where the target solution/suspension contacts the anti-solvent is preferably less than 20 °C, more preferably less than 10 °C, most preferably less than 5 °C, such as about 0 °C or between 2 and –2 °C. It is preferably no less than –5 °C, more preferably no less than 0 °C.

To ensure that the anti-solvent fluid is at a temperature of 25 °C or below at the point of its contact with the target solution/suspension, it is typically necessary both for the anti-solvent to be at 25 °C or below immediately before it contacts the target solution/suspension, and also for the temperature to be 25 °C or below in the region (typically within a particle formation vessel) in which the fluids contact one another and particle formation takes place.

The invention can provide particular advantages in the production of activated PAGs and PAG conjugates, since it provides a one-step process by which the materials may either be precipitated from solution (for instance, from the solution in which they were synthesised) or even directly prepared in particulate form. In most cases the need for a subsequent drying step and/or additional purification steps will be eliminated. There are clear advantages to the use of a one-step process, notably the potential for higher overall yields, greater processing efficiency and lower risks of contamination. Other advantages stemming from the use of a GAS-, in particular a SEDS™-, type process can include the ability to achieve high purity (in particular with respect to residual solvent levels), dry products with good handling properties and a high degree of control

over the physicochemical aspects of the particles formed, for instance their size, size distribution and morphology. The GAS/SEDS™ process also avoids the need for high levels of organic solvents, which can present waste handling and environmental problems, require high processing capacities and contaminate the end product.

In the method of the invention, the anti-solvent fluid is preferably a fluid which would be gaseous at atmospheric pressure. In other words, it preferably has a vapour pressure above 1 bar at ambient temperature (eg, at 22 °C). It is preferably used in liquid form, and is more preferably above its critical pressure P_c under the chosen operating conditions.

Suitable anti-solvents include nitrogen, nitrous oxide, sulphur hexafluoride, xenon, ethylene, dimethyl ether, chlorotrifluoromethane, ethane, propane, trifluoromethane and noble gases such as helium, neon or argon. The anti-solvent may comprise a mixture of two or more fluids, so long as the overall mixture is at the appropriate temperature and pressure.

The anti-solvent is preferably CO_2, more preferably liquid CO_2 such as at a pressure of from 75 to 125 bar.

The anti-solvent fluid may optionally contain one or more modifiers (cosolvents), for example water, methanol, ethanol, isopropanol or acetone, which change its ability to dissolve other materials. When used, a modifier preferably constitutes not more than 40 mole %, more preferably not more than 20 mole %, and most preferably between 1 and 10 mole %, of the anti-solvent fluid.

The anti-solvent (together with any modifiers it contains) must be miscible or substantially miscible with the fluid vehicle at the point of their contact, to allow extraction of the vehicle from the target solution/suspension. By "miscible" is meant that the two fluids are miscible in all proportions, and "substantially miscible" encompasses the situation where the fluids can mix sufficiently well, under the operating conditions used, as to achieve the same or a similar effect, ie, dissolution of the fluids in one another and precipitation of the target substance. However the anti-solvent must not to any significant extent, at the point of particle formation, extract or dissolve the target substance. In other words, it should be chosen so that the target substance is for all practical purposes (in particular, under the chosen operating conditions and taking into account any modifiers present) insoluble or substantially insoluble in it. Preferably the target

substance is less than 10^{-3} mole %, more preferably less than 10^{-5} mole %, soluble in the anti-solvent fluid.

The vehicle is a fluid which is able to carry the target substance in solution or suspension, the term "suspension" including colloidal systems such as emulsions. It may comprise a mixture of two or more component fluids. It may contain, in solution or suspension, other materials apart from the target substance, examples including surfactants, stabilisers, buffers and solubilisers.

The vehicle is preferably a liquid. It is preferably a solvent for the target substance, so that the target substance is carried in solution rather than suspension. The target substance preferably has a solubility (at ambient temperature) of 10^{-4} mole % or greater in the vehicle, more preferably 10^{-3} mole % or greater – typical PAGs, in particular PEGs, for use in the invention may for example have an aqueous solubility of 30 to 40 % w/v or greater.

Suitable vehicles for PAGs, PAG derivatives and PAG conjugates are organic solvents such as ketones (eg, acetone), halohydrocarbons (eg, dichloromethane (DCM) or chloroform), acetonitrile, tetrahydrofuran (THF) and mixtures thereof. It is preferred that the vehicle be incapable of hydrogen bonding to any significant extent with the ether groups of the PEG; thus for instance it may be preferred for the vehicle not to comprise fluids such as alcohols, dimethyl formamide (DMF) and dimethyl sulphoxide (DMSO).

Water and aqueous solvents may also be suitable, particularly for instance for conjugates of PAGs with water soluble active substances such as proteins. However since water is immiscible with the preferred anti-solvent liquid CO_2, an aqueous vehicle generally necessitates the use of an alternative, water-miscible anti-solvent and/or of an anti-solvent modifier and/or of an additional fluid vehicle as described below.

Moreover for an aqueous vehicle, operating temperatures close to 0 °C may cause the formation of ice crystals and it may therefore be preferred to operate at higher temperatures, for instance at 5 °C or above. Alternatively, again the use of an additional organic solvent vehicle, for instance acetone, may in cases mitigate this problem, especially when the organic vehicle is used in excess.

The concentration of the target substance in the vehicle may suitably be from 0.5 to 40 % w/v, preferably from 1 to 25 % w/v, depending on the natures of the target substance and vehicle. The concentration should be chosen to ensure an appropriate viscosity in the target solution/suspension, to facilitate its contact with the anti-solvent fluid during the GAS process.

Often it is desirable to use the minimum possible amount of the vehicle to solvate or suspend the target substance, preferably so as to create a single phase solution.

The target solution/suspension may effectively comprise two or more fluids, which may be pre-mixed but which are preferably mixed *in situ* either substantially simultaneously with or immediately before their contact with the anti-solvent. Such systems are described, eg, in WO-96/00610 and WO-01/03821. The first fluid vehicle may carry the target substance and may for example be water. The second fluid vehicle (which may for example be an organic solvent such as an alcohol or ketone) may be introduced in the anti-solvent flow or separately to the other fluids. It is preferably also introduced in such a manner that it can be dispersed and extracted by the anti-solvent fluid at the same time as the first vehicle, and ideally also in a manner such that the kinetic energy of the anti-solvent can aid in mixing the first and second vehicles. Such fluid contact may be achieved for example using a multi-component coaxial nozzle of the type described in WO-96/00610.

The second vehicle may be a nonsolvent for the target substance, by which is meant that the target substance would typically have a solubility in the second vehicle of less than 10^{-3} mole %, preferably less than 10^{-5} mole %. This can help to induce precipitation of the target substance when the second vehicle contacts the target solution/suspension. For instance, the first vehicle may be acetone, tetrahydrofuran or dichloromethane and the second (nonsolvent) vehicle cyclohexane. To further aid precipitation, the second vehicle may contain a "seed" of the target substance, or indeed of any other suitable material (insoluble in the second vehicle), to help induce nucleation of the target substance when the second vehicle comes into contact with the target solution/suspension.

When carrying out this version of the invention, the second vehicle and the target solution/suspension preferably contact one another immediately before their contact with the anti-solvent fluid. The target solution/suspension should also, generally, be highly saturated.

Where two or more fluid vehicles are used, they are preferably miscible or substantially miscible with one another and at least one of them should be miscible with the anti-solvent fluid as described above. The anti-solvent-miscible vehicle(s) are suitably present in excess (relative to the amount of other vehicles present) at the point of anti-solvent contact, so that the anti-solvent can extract all the vehicles together. This may apply for instance if one or more of the vehicles is less than substantially soluble in the anti-solvent fluid (for instance, it has a solubility of 2 or even 1 mole % or less in the anti-solvent).

An excess of one vehicle over another may be achieved by appropriate selection of vehicle flow rates. It can also be desirable for instance when two vehicles are less than fully miscible with one another. Where for example two vehicles are used, the molar ratio of the first to the second may be less than 1:1.5 (for instance, between 1:100 and 1:1.5), preferably less than 1:4, more preferably less than 1:6, most preferably less than 1:9 or 1:10 or 1:20.

Generally speaking however the molar ratio of two vehicles at the point of particle formation may range between 1:99 and 99:1. The relative amounts of all vehicles at this point must of course be chosen so that they are extractable, *together*, into the anti-solvent fluid. Ideally the amounts are chosen so that, under the operating conditions used, the vehicles form a single phase mixture at the point of particle formation.

Where two or more fluid vehicles are used, they may carry two or more target substances, to be combined in some way (for instance, co-precipitated as a matrix, or one precipitated as a coating around the other, or precipitated as the product of an *in situ* reaction between the substances) at or before the point of particle formation. Target substance(s) may also be carried in the anti-solvent fluid.

When carrying out the method of the invention in a particle formation vessel, the temperature and pressure inside the vessel are ideally controlled so as to allow particle formation to occur at or substantially at the point where the target solution/suspension meets the anti-solvent fluid. The conditions in the vessel must generally be such that the anti-solvent fluid, and the solution which is formed when it extracts the vehicle, both remain in a compressed state whilst in the vessel. At the time of particle formation, there should be a *single-phase* mixture of the vehicle and the anti-solvent fluid, to prevent the particulate product being distributed between two or more fluid phases, in some of which it might be able to redissolve.

Selection of appropriate operating conditions will be influenced by the natures of the fluids involved (in particular their solubility and miscibility characteristics) and also by the characteristics desired of the particulate end product, for instance yield, particle size and size distribution, purity and morphology. Variables include the flow rates of the anti-solvent fluid and the target solution/suspension, the concentration of the target substance in the vehicle, the temperature and pressure inside the particle formation vessel, the anti-solvent temperature upstream of the vessel and the geometry of the fluid inlet(s) into the vessel. The method of the invention preferably involves controlling one or more of these variables so as to influence the physicochemical characteristics of the particles formed.

The flow rate of the anti-solvent fluid relative to that of the target solution/suspension, and its pressure and temperature, should be sufficient to allow it to accommodate the vehicle at the point of fluid contact. The anti-solvent flow rate will generally be higher than that of the target solution/suspension – typically, the ratio of the target solution/suspension flow rate to the anti-solvent flow rate (both measured at or immediately prior to the two fluids coming into contact with one another) will be 0.001 or greater, preferably between 0.01 and 0.2, more preferably between about 0.03 and 0.1.

Thus, the anti-solvent flow rate will generally be chosen to ensure an excess of the anti-solvent over the vehicle when the fluids come into contact, to minimise the risk of the vehicle re-dissolving and/or agglomerating the particles formed. At the point of extraction of the vehicle it may constitute between 1 and 80 mole %, preferably 50 mole % or less or 30 mole % or less, more preferably between 1 and 20 mole % and most preferably between 1 and 5 mole %, of the resultant fluid mixture.

Both the anti-solvent and the target solution/suspension are ideally introduced into the particle formation vessel with a smooth, continuous and preferably pulse-less or substantially pulse-less flow. Conventional apparatus may be used to ensure such fluid flows.

The method of the invention preferably additionally involves collecting the particles following their formation, more preferably in a particle formation vessel into which the fluids are introduced.

According to a second aspect of the present invention, there is provided a method for forming particles of a target substance which consists essentially of one or more polyalkylene glycols (PAGs) or derivatives or conjugates thereof (including mixtures of these), which method comprises carrying out a GAS process, preferably a SEDS™ process, on a solution or suspension of the target substance in a fluid vehicle (the "target solution/suspension").

To our knowledge, although GAS has been used to co-precipitate PAGs (in particular PEGs) with other materials (for instance, as excipients for pharmaceutically active substances), it has not previously been used to precipitate PAGs alone, presumably because it was believed inappropriate for such temperature sensitive materials.

In this context, "consists essentially of" means that the target substance is either a PAG or PAG derivative/conjugate (or a mixture of such materials) with no other substances present, or contains greater than 80 % w/w, preferably greater than 90 % w/w, more preferably greater than 95 % w/w or 98 % w/w, of PAGs/PAG derivatives/PAG conjugates.

Preferred features of this second aspect of the invention, for instance with respect to reagents and operating conditions, may be as described above in connection with the first aspect of the invention. In particular, it preferably involves carrying out a GAS-type or SEDS™-type process but using as the anti-solvent a fluid, preferably a compressed fluid, which at the point of its contact with the target solution/suspension is at a temperature of 25 °C (298 K) or below. The method is preferably applied to a PAG or to an "activated" PAG of the type described above (or to a mixture of PAG and activated PAG). It may in particular be applied to a PEG, a PEG derivative or conjugate or a mixture of such materials.

By "a GAS-type process" in this regard is meant a process which involves contacting the target solution/suspension with a compressed fluid anti-solvent under conditions which allow the anti-solvent to extract the vehicle from the target solution/suspension and to cause particles of the target substance to precipitate from it, as described above, but using as the anti-solvent a fluid which is at a temperature of 25 °C or below, and therefore not necessarily supercritical or near-critical, at the point of its contact with the target solution/suspension.

By "a SEDS™-type process" is meant a process which involves the co-introduction of the target solution/suspension and the anti-solvent fluid into a particle formation vessel, under conditions

which allow the anti-solvent to disperse the target solution/suspension, and simultaneously to extract the fluid vehicle from it so as to cause particle precipitation, but using as the anti-solvent a fluid which is at a temperature of 25 °C or below, and therefore not necessarily supercritical or near-critical, at the point of its contact with the target solution/suspension. Suitable forms of fluid co-introduction are described for example in WO-95/01221, WO-96/00610, WO-98/36825, WO-99/44733, WO-99/59710, WO-00/67892, WO-01/03821 and WO-01/15664, in our co-pending PCT patent application no. PCT/GB PCT/GB01/04873 and in our co-pending UK patent application no. 0117696.5), although in connection with supercritical or near-critical fluid anti-solvents.

When carrying out the methods of the invention, the target substance may in general be, or be suitable or intended for use in or as, a pharmaceutical, nutriceutical or its excipient; a dyestuff; a cosmetic; a foodstuff; a coating; an agrochemical; a product of use in the ceramics, explosives or photographic industry; etc... It is preferably soluble or substantially soluble in the fluid vehicle, preferably having a solubility in it of 10^{-4} mole % or greater under the conditions under which the target solution is prepared (ie, upstream of the point of particle formation).

In a particularly preferred embodiment of the invention, the target substance is for use in or as a pharmaceutical or pharmaceutical excipient, or in or as a nutriceutical or nutriceutical excipient.

The target substance may be in a single or multi-component form (eg, it could comprise an intimate mixture of two materials, or one material in a matrix of another, or one material coated onto a substrate of another, or other similar mixtures). It may comprise two associated substances, for example in a covalently bonded conjugate such as the PAG-active substance complexes described above.

The methods of the invention may be used selectively to precipitate one or more target substances from a mixture of substances. The operating temperature and pressure, and the vehicle and anti-solvent, may be chosen so that only the target substance(s) precipitate whereas the other components of the mixture are extracted into the anti-solvent with the vehicle; this depends naturally on the solubility of the target substance(s) in the vehicle and anti-solvent under the chosen operating conditions.

Thus, the present invention may be used for example to purify a substance in a mixture containing that and other substances. It may be used to fractionate mixtures of substances such as of different molecular weight polymers.

In particular, it may be used selectively to precipitate a target "activated" PAG from a reaction mixture containing the target as well as impurities such as unreacted PAG base polymer. It may be used selectively to precipitate a PAG-active substance conjugate from a reaction mixture which also contains, for instance, unconjugated starting materials. It may be used to fractionate mixtures of PAGs and PAG derivatives/conjugates of different molecular weights, since polymer molecular weight can significantly influence solubility in both fluid vehicles and anti-solvent fluids.

The methods of the invention may be used to coformulate two or more target substances to produce a multi-component product. Such a product may for example comprise an intimate mixture of two or more materials, such as in a solid dispersion, or one material coated onto a substrate of another, or a mixture of such physical forms. Examples include two pharmaceuticals intended for co-administration, or a pharmaceutical together with an excipient.

The two or more target substances may be co-introduced in a common fluid vehicle, or in separate fluid vehicle(s) which contact one another at or immediately before the point of anti-solvent contact. Target substance(s) may also be carried in the anti-solvent itself.

In particular, the methods of the invention may be used to coformulate an active substance, such as a pharmaceutically or nutriceutically active substance, with a PAG or PAG derivative.

Within the coformulated product, the target substances may be associated with one another for instance via some form of chemical or physical interaction. In particular they may be associated in the form of a complex or conjugate or other reaction product. Alternatively, they may be present as distinct entities.

According to a third aspect of the invention, there is provided a particulate product formed using a method according to the first or the second aspect. This may in particular consist essentially of a PAG or PAG derivative or PAG conjugate.

A PAG or PAG derivative/conjugate formed using a method according to the present invention, in particular a PEG or PEG derivative/conjugate, can often have good physicochemical particle properties. It may for instance be produced in the form of a dry powder, usually easily handlable and free flowing and with good shelf storage stability. Small particles, for instance of mass median diameter 30 μm or less, preferably 20 μm or less or even 10 μm or 5 μm or less, may be produced, and with a relatively narrow size distribution (for instance with a particle size spread of less than 2.4, such as from 1.5 to 2.4, preferably less than 2). (Particle size "spread" is defined as $(D_{90} - D_{10}) / D_{50}$ where D is the diameter of the relevant particle population.) In cases, particle sizes may range from 1 to 15 μm, preferably from 2 to 12 μm, more preferably from 4 to 10 μm.

Typically the methods of the invention may be used to reduce the particle size of the target substance to 90 or 80 or 70 % or less of that of the starting material, preferably to 50 % or less of that of the starting material, more preferably to 40 % or 30 % or 20 % or even 10 % or less.

Particle sizes may be measured for instance using (a) an Aerosizer™ time-of-flight instrument (which gives an aerodynamic equivalent particle diameter, MMAD) or (b) a laser diffraction sensor such as the Helos™ system available from Sympatec GmbH, Germany (which provides a geometric projection equivalent MMD). Volume mean diameters may be obtained in both cases using commercially available software packages.

Particles produced according to the invention also tend to possess better dissolution properties (ie, a greater speed and/or efficiency of dissolution) in aqueous solutions than corresponding products made by prior art techniques. In particular PEGs and PEG derivatives/conjugates produced by prior art techniques such as freeze drying often suffer from poor dissolution characteristics, which can present problems if (as is likely where a PEG is to be used as an excipient for instance for an injectable pharmaceutical) they need to be formulated in high concentrations.

In the case where the product is a conjugate of a PAG or PAG derivative with an active substance, in particular a PEG conjugate, it preferably has an aqueous solubility of at least 300 mg/ml, more preferably at least 400 mg/ml, at room temperature.

The product of the present invention preferably contains less than 500 ppm, more preferably less than 200 ppm, most preferably less than 150 or 100 ppm residual solvent, by which is meant solvent(s) which were present at the point of particle formation, for instance in the target

solution/suspension and/or the anti-solvent fluid. Still more preferably the product contains no detectable residual solvent, or at least only levels below the relevant quantification limit(s). Generally it preferably contains less than 2.5 % w/w, more preferably less than 2 or 1.5 or 1 % w/w of impurities, by which is meant substances (either solid or liquid phase) other than the target substance intended to be formed into particles.

Where the product contains an active, in particular a pharmaceutically active, substance and more particularly a proteinaceous material, the active substance preferably retains at least 90 %, more preferably at least 95 %, of its original activity after processing into particles using the method of the invention, or regains that activity on resolvation. "Original activity" may here mean the activity of the protein alone, ie, unconjugated, in the absence of excipients and prior to SEDS™ processing, and/or in the case where the target substance is a PAG-protein conjugate, the activity of the protein within the conjugate prior to the SEDS™ processing.

The active substance preferably also retains, or substantially retains, its chemical integrity following SEDS™ processing, which may include its structural properties in particular where it has a secondary and/or tertiary structure as in the case of proteinaceous active substances.

Because embodiments of the present invention are modified versions of the inventions disclosed in WO-95/01221, WO-96/00610, WO-98/36825, WO-99/44733, WO-99/59710, WO-01/03821 and WO-01/15664, and of those disclosed in our co-pending PCT patent application no. PCT/GB PCT/GB01/04873 and our co-pending UK patent application no. 0117696.5, technical features described in those documents, for instance regarding the selection of appropriate reagents, operating conditions and processing apparatus, can apply also to the present invention. The nine earlier documents are therefore intended to be read together with the present application.

In this specification the term "substantially", when applied to a condition, is meant to encompass the exact condition (eg, exact simultaneity) as well as conditions which are (for practical purposes, taking into account the degree of precision with which such conditions can be measured and achieved) close to that exact condition, and/or which are similar enough to that exact condition as to achieve, in context, the same or a very similar effect. In particular, "substantially simultaneously" and "substantially immediately", referring to the timing of fluid contact events, imply sufficiently small time intervals (for instance, between the anti-solvent fluid contacting the vehicle(s), and the fluids entering a particle formation vessel) as preferably to eliminate, or

substantially eliminate, the risk of particle formation occurring upstream of the particle formation vessel. Where two vehicles are to be mixed immediately before their contact with the anti-solvent fluid, the timing of their contact with one another, relative to that of their dispersion by the anti-solvent, will depend on the nature of the fluids (in particular the degree of immiscibility of the two vehicles), the target substance and the desired end product, as well as on the size and geometry of the particle formation vessel and the fluid inlet and on the fluid flow rates. The contact may occur within about 0.001-10 seconds, more preferably within about 0.01-5 seconds, most preferably within about 0.01-1 second, of the dispersion.

References to solubilities and miscibilities, unless otherwise stated, are to the relevant fluid characteristics under the operating conditions used.

References to "operating conditions" are to the conditions (including temperature, pressure and the natures and amounts of the fluids (including modifiers) and other reagents present) under which particle formation occurs, ie, generally the conditions under which the target solution/suspension contacts the anti-solvent fluid, which is preferably at or substantially at the point where the fluids enter the vessel in which particle formation is to take place.

The present invention will now be illustrated by means of the following examples.

Detailed description

These examples, which relate to the precipitation of PEGs, activated PEGs, PEG conjugates and PEG/PPG copolymers, were carried out using a SEDS™ particle formation system of the general type shown schematically in Fig 1 of WO-95/01221. A two- or in some cases three-passage coaxial nozzle (see Figs 3 and 4 of WO-95/01221) was used to co-introduce into a particle formation vessel (i) a solution of the target substance in a fluid vehicle, (ii) liquid CO_2 and in some cases (iii) a second fluid vehicle. The temperature and pressure were controlled within the vessel to ensure the CO_2 remained in liquid form throughout the process. Particle formation occurred at the nozzle outlet, where the fluids came into contact immediately before entering the vessel; here the CO_2, introduced with a much higher flow rate than that of the target solution, acted both to disperse and to mix the fluids and also to extract the vehicle(s) thus precipitating the target substance.

The operating pressure within the vessel was 100 bar in all experiments. The operating temperature (ie, that of the CO_2 on entering the particle formation vessel, and also within the vessel itself) was in most cases 25 °C; similar if not better results could be expected at lower temperatures.

The outlet diameter of the nozzle used was, unless otherwise stated, 200 μm. The CO_2 flow rate (measured at the pump head) was generally 20 ml/min, that for the target solution generally 0.1 ml/min.

The solvents used were acetone, tetrahydrofuran (THF), dichloromethane (DCM) and ethyl acetate. In certain cases, cyclohexane was used as a second (anti-solvent) vehicle. Wherever possible, the target solutions were made up in saturated form.

Since many PEGs have a tendency to gel in solution in organic solvents if not kept warm, especially if saturated, the PEG target solutions were maintained where possible at between 25 and 35 °C. To mitigate problems caused by high target solution viscosity, in cases a stainless steel loop of volume between 3 and 29 ml was inserted between the pump outlet and the oven inlet for the solution; this loop was pre-loaded manually with the target solution and on commencement of the particle formation run, neat solvent was pumped through the loop thus forcing the target solution into the pressure vessel.

All particle sizes and spreads were measured using an Aerosizer™, unless otherwise stated.

All PEGs, PEG derivatives and PEG conjugates used were sourced from Shearwater Corporation, Huntsville Alabama, US.

Examples 1 demonstrate the formation of particulate PEGs using a method according to the present invention. In Examples 2 and 3, "activated" PEG derivatives are precipitated by the same method. Examples 4 to 6 show the precipitation of PEG-active substance conjugates in accordance with the invention. Examples 7 show the precipitation of a PEG/PPG copolymer, Examples 8 the precipitation of a range of PEG-protein conjugates.

Examples 1

Linear PEGs of formula (I) (R^1 = methyl), of different molecular weights, were successfully precipitated using a SEDS™-type process according to the method of the invention.

The 5 kDalton (± 500 dalton) PEG starting material had a particle size of 27.3 μm and a melting point of 64 °C and contained ≤ 1 % of the non-methylated base diol. The 12 kDalton (± 1200 dalton) starting material had a particle size of 35.1 μm and a melting point of 65.7 °C and contained ≤ 1.5 % of the non-methylated diol. The 20 kDalton (±200 dalton) PEG starting material had a particle size of 46.9 μm, a melting point of 66.6 °C and a diol content of ≤ 3 %.

The CO_2 flow rate was 20 ml/min (measured at the pump head), that for the target solution 0.1 ml/min. A 50 ml particle formation vessel was used.

Different solvents and target solution concentrations were tested, as set out in Table 1 below, together with the product yields and particle sizes. In all cases the product was a fine free-flowing powder with a relatively narrow particle size distribution.

Table 1

Experiment no.	*PEG molecular weight (kDaltons)*	*Solvent*	*PEG solution concentration (% w/v)*	*Yield (%)*	*Product particle size (μm)*	*Particle size spread*	*Run duration (min)*
1.1	5	Acetone	6	73	4.8	1.5	36
1.2	5	THF	7.5	71	11.6	1.7	32
1.3	12	Acetone	6	59	5.9	1.6	35
1.4	12	THF	7.5	82	6.3	1.6	110
1.5	20	Acetone	15	93	7.863	2.3	80
1.6	20	DCM/cyclohexane	6	67	5.366	2	70

Experiment no.	*PEG molecular weight (kDaltons)*	*Solvent*	*PEG solution concentration (% w/v)*	*Yield (%)*	*Product particle size (μm)*	*Particle size spread*	*Run duration (min)*
		mixture (45:55)					
1.7	20	DCM	33	60	17.11	1.8	30

In a further experiment, no. 1.8, the 20 kDalton PEG was precipitated from THF (10 % w/v) in the presence of cyclohexane. The target solution and the cyclohexane were co-introduced into the particle formation vessel via separate passages of the three-component coaxial nozzle, so as to contact one another only immediately before their contact with the CO_2. The cyclohexane flow rate was 0.5 ml/min and the run duration 70 minutes; all other operating conditions were the same as for Experiments 1.1 to 1.7. In this case the product yield was 72.2 %, and its particle size was 4.665 μm (Sympatec™ particle size 4.7 μm) with a spread of 1.8.

Examples 2

These experiments demonstrate the precipitation, using a SEDS™-type method according to the invention, of an "activated" PEG. The starting material was a linear PEG of formula (II) in which R^1 = methyl and X = CHO (at least 80 % aldehyde groups, ie, ≤ 20 % unactivated PEG). It had a molecular weight of 30 kDaltons (± 3,000 dalton), a particle size of 55.6 μm and a melting point of 59.9 °C. It contained residual solvents DCM and isopropyl alcohol (IPA) in total amounts less than 100 ppm.

Attempts to precipitate this material from methanol, a methanol/ethanol (1:1) mixture and dichloromethane at supercritical temperatures (80, 60 and 40 °C) did not yield a particulate product, presumably due to the solubility of the PEG in the CO_2 anti-solvent under such conditions. In some cases this resulted in no yield at all, in others the PEG deposited in the form of a waxy as opposed to a fine particulate solid.

The activated PEG was however successfully precipitated from various solvent systems using an operating temperature of 25 °C and an operating pressure of 100 bar, as shown in Table 2 below. The nozzle outlet diameter was 200 μm for all except Experiment 2.6, in which it was 500 μm. The vessel volume was 50 ml for Experiments 2.1 to 2.4, and 500 ml for Experiments 2.5 to 2.8.

Where two vehicles were used, they were introduced via separate passages of a three-component coaxial nozzle, as described in connection with Experiment no. 1.8.

Table 2 also shows the yields and particle sizes for the products, all of which were in the form of fine free flowing powders with narrow particle size distributions.

Table 2

Expt no.	*1st vehicle*	*2nd vehicle*	*PEG solution conc^n (% w/v)*	*PEG solution flow rate (ml/min)*	*2nd vehicle flow rate (ml/min)*	*CO_2 flow rate (ml/min)*	*Yield (%)*	*Product particle size (μm)*	*Particle size spread*	*Run duration (min)*
2.1	#A	–	1	0.1	–	20	64	3.076	1.7	110
2.2	#B	–	15	0.1	–	20	62	7.128	1.9	90
2.3	#B	#C	15	0.1	1	20	64	10.01	1.7	66
2.4	#D	#C	5	0.1	0.5	30	99	12.2	2	300
2.5	#D	#C	5	0.2	0.5	20	94	8.31	2.1	150
2.6	#A	–	15	0.1	–	20	73	16.1	1.6	300

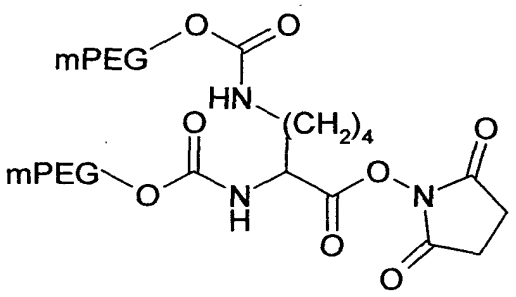
(Key to vehicles: #A = acetone; #B = DCM; #C = cyclohexane; #D = DCM/acetone mixture (1:49 v/v)).

Experiment no. 2.1 was repeated (run duration 80 minutes) at an operating temperature of 0 °C, and again a particulate product was successfully obtained, having a particle size of 33 μm and size spread of 1.7. The yield was 62 %.

Residual solvent levels were measured for the products of Examples 2.4 and 2.5. In both cases, no DCM or cyclohexane was detected, and the acetone levels were below the 150 ppm quantification limit.

Examples 3

In these experiments, a SEDS™-type process was used to precipitate a branched two-arm activated PEG of formula (V) below:



(V)

in which mPEG represents a linear PEG chain as defined in formula (I) with R^1 being methyl and –O-R^2 being replaced by the N-hydroxysuccinimidyl ester/amide linkage group. This is a branched PEG of which the two mPEG arms are connected via urethane linkages to a lysine linker activated as the N-hydroxysuccinimide ("NHS") ester, ie, mPEG2-N-hydroxysuccinimide.

The activated PEG has a total molecular weight of 40 kDaltons (20 kDaltons for each mPEG arm). Its "apparent" molecular weight is expected to reflect that of each arm, ie, in this case 20 kDaltons (± 4,000 dalton). The starting material had a particle size of 33.5 μm and a melting point of 60.9 °C, and contained the residual solvents DCM and IPA in total amounts less than 100 ppm.

It was precipitated from DCM (15 % w/v; solution flow rate 0.1 ml/min). Cyclohexane was co-introduced (flow rate 1 ml/min) with the PEG solution and CO_2 via a three-component nozzle, as described in connection with Experiment 1.8 above.

The operating temperature was 25 °C and the pressure 100 bar. The nozzle outlet diameter was 500 μm and the vessel volume 500 ml. The run duration was 120 minutes.

The experiment resulted in a free flowing powder, with a yield of 63 %. The product particle size was 18.81 μm (spread 1.7).

Examples 4

A conjugate of mPEG (ie, a linear PEG chain as defined in formula (I) in which R^1 = methyl) with dipalmitoyl phosphatidyl ethanolamine (DPPE) was precipitated from ethyl acetate using the method of Examples 1 to 3. The structure of the ethanolamine-mPEG link was :

$$-CH_2-CH_2-NH-CO_2-mPEG.$$

The molecular weight of the PEG was 5 kDaltons, that of the conjugate 5750 ± 600 daltons. The particle size of the starting material was 8.9 μm and its melting point 54 °C. It had a purity of > 97 % and contained the residual solvents chloroform, acetonitrile and IPA in total amounts less than 100 ppm.

The PEG solution concentration was 2.5 % w/v. Its flow rate into the particle formation vessel was 0.1 ml/min and the CO_2 flow rate was 20 ml/min. The operating temperature was 25 °C and the pressure 100 bar. The nozzle outlet diameter was 200 μm, the vessel volume 50 ml.

In Experiment 4.1, using a run duration of 30 minutes, a fine free flowing powder was obtained with 41 % yield. The particle size was 7.6 μm. In Experiment 4.2, using a run duration of 132 minutes, a fine free flowing powder was obtained with 56 % yield. Here the particle size was 9.0 μm, spread 1.9.

Attempts to precipitate the PEG conjugate at 35 °C, all other conditions the same, were unsuccessful.

Example 5

A conjugate of mPEG (ie, a linear PEG chain as defined in formula (I) in which R^1 = methyl) with distearoyl phosphatidyl ethanolamine (DSPE) was precipitated from DCM using the method of Examples 1 to 3. The structure of the DSPE-mPEG link was as for the conjugate of Example 4. The molecular weight of the PEG was 2 kDaltons. The particle size of the starting material was 12.3 μm and its melting point 50.6 °C. It had a purity of > 97 % and contained the residual solvents chloroform, acetonitrile and IPA in total amounts less than 100 ppm.

The PEG solution concentration was 2.5 % w/v, its flow rate 0.1 ml/min. The CO_2 flow rate was 20 ml/min. The operating temperature was 25 °C and the pressure 100 bar. The nozzle outlet diameter was 200 μm, the vessel volume 50 ml. The run duration was 30 minutes.

A fine free flowing powder was obtained with 52 % yield. The particle size was 7.6 μm, standard deviation 2.1.

Again attempts to precipitate the conjugate at 35 °C were unsuccessful.

Example 6

An ester-linked conjugate of mPEG (ie, R^1 = methyl) with R-*trans*-retinoic acid (ATRA) was precipitated from acetone using the method of Examples 1 to 3. The molecular weight of the PEG was 5 kDaltons. The particle size of the starting material was 20.0 μm (standard deviation 1.9), its melting point 54.7 °C.

The conjugate solution concentration used was 5 % w/v, its flow rate 0.1 ml/min. The CO_2 flow rate was 20 ml/min. The operating temperature was 25 °C and the pressure 100 bar. The nozzle outlet diameter was 200 μm, the vessel volume 50 ml. The run duration was 30 minutes.

A fine free flowing powder was obtained with 33 % yield. The particle size was 7.38 μm, standard deviation 1.83.

In a further experiment, using a run duration of 120 minutes but with all other operating conditions the same, a particulate product was obtained in a yield of 78 %, particle size 5.92 μm, standard deviation 1.55.

Using supercritical processing temperatures (ie, above 31 °C) did not result in successful precipitation of the PEG conjugate.

Example 7

A commercially available PEG/PPG copolymer, Pluronic™ F127 (average molecular weight 12.6 kDaltons) was precipitated from a 1:9 (v/v) DCM/acetone mixture. Pluronic™ F127 contains 70 % PEG and 30 % PPG. The starting material had a melting point of 56 °C.

The Pluronic™ solution concentration used was 5 % w/v, its flow rate 0.4 ml/min. The operating temperature was 25 °C and the pressure 100 bar. The nozzle outlet diameter was 200 μm, the vessel volume 50 ml.

In Experiment 7.1 the CO_2 flow rate was 20 ml/min; in Experiment 7.2 it was 10 ml/min.

Fine free flowing powders were obtained, with yields of 68 % and 66 % for Experiments 7.1 and 7.2 respectively.

Examples 8

Conjugates of the protein lysozyme with linear mPEGs of various molecular weights were processed from aqueous solution using the method of the invention. All conjugates were obtained from Shearwater Corporation, Huntsville Alabama, US.

The operating temperature was 0 °C and the pressure 100 bar. Each target solution contained 20 mg/ml conjugate. Acetone was used, as in Example 1.8, as a second (non-solvent) vehicle. The flow rates were 0.03 ml/min for the target solutions, 5 ml/min for the acetone and 20 ml/min for the liquid CO_2 anti-solvent.

Unconjugated lysozyme was also subjected to SEDS™ processing, again from a 20 mg/ml aqueous solution in the presence of acetone, using the same operating conditions as for the PEG conjugates.

The results are shown in Table 3. Particle sizes are by Aerosizer™.

Table 3

Experiment no.	*mPEG molecular weight (kDaltons)*	*Yield (%)*	*Particle size (μm)*	*Starting material particle size (μm)*
8.1	N/A (unconjugated lysozyme)	20	14	17.8
8.2	5	67	16.5	7.9
8.3	20	42	14.3	13.4
8.4	40	28	16.5	18.7

SEM observation of the SEDS™ products revealed them to be smooth approximately spherical particles as compared to rough and broken plate-like particles for the conjugate starting materials.

The SEDS™-produced lysozyme was found to have retained 105 % of its original (ie, pre-SEDS™) activity, and the 5 kDalton PEG-lysozyme conjugate 108 %.

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